REMARKS

Applicants are submitting this supplemental amendment and response as a supplement to the response filed December 11, 2008. Applicants request this response be considered and that the response filed December 11, 2008 be disregarded.

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Applicants have amended claim 11 and added new claim 12.

The rejection of claims 1-6, and 11 under 35 U.S.C. 112, second paragraph, as being indefinite are respectfully traversed.

Applicants respectfully point out that claim 11 is directed to a method of producing polyunsaturated fatty acids, and not to a method of extracting or isolating such polyunsaturated fatty acids. On page 2 of the Office Action, the Examiner acknowledges that "*there is no specific requirement which specifically addresses the need for a recovery step*". Applicants believe that the Examiner's request will unnecessarily leave some area open between what is disclosed by the invention and what is covered by the claims. Thus, narrowing the scope of claim 11 to include a recovery step may render the patent just as worthless as if it were invalid, since anyone would be free to use the invention in the unprotected area. The claims satisfy 35 U.S.C. 112, second paragraph as they particularly point out and distinctly claim a method for producing polyunsaturated fatty acids from diatomaceous algae. Recovery is not an essential step. If recovery were desired, a person skilled in the art would know how to recover the produced polyunsaturated fatty acids following well-known techniques, which techniques are taught, for example, in:

> D'Souza et al., 2002, "Flocculated microalgae concentrates as diets for larvae of the tiger prawn Penaeus monodon Fabricius", Aquacult. Nutr., 8: 113-120;

Babarro et al., 2001, "Influence of preservation techniques and freezing storage time on biochemical composition and spectrum of fatty acids of *Isochrysis galbana* clone T-ISO", Aquacult. Res. 32: 565-572; and

Heasman et al., 2000), "Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs - a summary", Aquac. Res. 31; and

Knuckey et al., 2006, "Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds", Aquacultural Engineering 35: 300-313.

A copy of these references were submitted in an IDS field December 12, 2008. Therefore the rejection under 35 U.S.C. 112, second paragraph, as being indefinite is improper and should be withdrawn.

The rejection of claims 1, 3-5, and 11 under 35 U.S.C. 102(b), as anticipated by McGinnis are respectfully traversed.

Applicant respectfully points out that nowhere in McGinnis is there disclosed a method for specifically producing polyunsaturated fatty acids as claimed in the present application. McGinnis only teaches a method of increasing the lipid content of *C. muelleri*, without specifically demonstrating the increase of polyunsaturated fatty acids. McGinnis also points out (page 23, 2nd para.) that total lipid increases in certain diatoms but not in others. McGinnis specifically states that the nutrient stress is applied at day 4 (page 20, 2nd para.). According to figure 3 (page 21), day four is clearly in the **dormant phase** of growth, not in at the end of the exponential phase as stated by the Examiner in the office action. Thus, McGinnis does not teach the claimed invention, i.e., a method of specifically increasing polyunsaturated fatty acids by applying at least one growth-limiting factor to a culture of diatomaceous algae at the **end of the exponential growth phase**, causing growth arrest of said culture and production and stocking by said algae in culture of polyunsaturated fatty acids. Applicants wish to respectfully remind the Examiner that it is well known in the art of cell culture that the same stress applied to a cell culture at different time points on the growth curve will have dramatically different results. Applicants also respectfully remind the Examiner that lipids encompass a broad class of molecules such as fats, oils, waxes, cholesterol, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. Thus, demonstrating that

the lipid content is increased does not necessarily mean that the polyunsaturated fatty acid content is increased. For example, the content of vitamins might increase, which will translate in an increase of the total lipids amount, without affecting the amount of polyunsaturated fatty acids. Therefore the rejection under 35 U.S.C. 102(b) for anticipation by McGinnis is improper, especially in view of McGinnis' own admission that total lipid increases in certain diatoms but not in others (showing unpredictability), and should be withdrawn.

The rejection of claims 4, 5 and 11 under 35 U.S.C. 102(b), as anticipated by Dempster are respectfully traversed. Applicants respectfully submit that nowhere in Dempster is there disclosed a method for producing polyunsaturated fatty acids as claimed in the present application. Dempster only teaches a method of increasing the lipid content of *N. communis*, without demonstrating the increase of polyunsaturated fatty acids specifically. As pointed out above with respect to McGinnis, the nutrient stress of Dempster was applied after the exponential growth phase, during the dormant phase on Day 4 (page 19). Dempster does not teach a method for specifically producing polyunsaturated fatty acids from diatomaceous algae, comprising the step of applying at least one growth-limiting factor to a culture of diatomaceous algae at the end of the exponential growth phase, causing growth arrest of said culture and production and stocking by said algae in culture of polyunsaturated fatty acids. Thus, the claims are novel in view of Dempster. Therefore the rejection under 35 U.S.C. 102(b) for anticipation by Dempster is improper and should be withdrawn.

The rejection of claims 1, 3, 4 and 11 under 35 U.S.C. 102(b), as anticipated by Taguchi are respectfully traversed.

Applicants note that the Examiner cites Taguchi and alleges that "Taguchi teaches a process of culturing diatomaceous algae wherein at least some of the diatoms were in the exponential growth phase when growth-limiting factors, such as silica deprivation, were **applied**, causing growth arrest and the production of polyunsaturated fatty acids., see e.g., pages 33-42". The Examiner refers to pages 33-42 of Taguchi that is of record. However, the only Taguchi reference of record is the one that Applicants had submitted in an IDS, and this reference only contains pages 260-267. Applicant respectfully request that the Examiner quote the passages of Taguchi relied upon so that an appropriate response can be made.

Referring to Taguchi, of record, as a whole, Applicant respectfully point out that no growth limiting factors were applied and silica deprivation should in fact be read as silica *exhaustion* from the media. In one embodiment of the present invention, Applicants are applying the growth-limiting factor of silica deprivation, i.e. the silica has to be removed from the media or the media changed to remove any silica therefrom. Applicants respectfully submit that nowhere in Taguchi is there disclosed a method for producing polyunsaturated fatty acids as claimed in the present application. Taguchi only teaches a method of increasing the lipid content of *Chaetoceros gracilis, Hantzschia* sp. and *Cyclotella* sp., without demonstrating the increase of polyunsaturated fatty acids specifically. Additionally, as noted above, Taguchi applies the nutrient stress during stationary phase (page 261, para. 3). Figure 1 shows arrows indicating when the stress was applied, in each instance the stress was applied well into the dormant phase not at the end of the exponential growth phase, causing growth arrest of said culture and production and stocking by said algae in culture of polyunsaturated fatty acids, as claimed in the present application. Furthermore, if, according to Taguchi, the addition of silica is the stress itself, then it is not a growth-limiting stress because the addition of silica will induce cell multiplication and cell growth, whereas, in the present invention, the stress is a growth-limiting factor. Therefore, Taguchi only teaches that, first of all, to have cell division and cell growth, it is necessary to add silica regularly to the culture, avoiding the stationary phase. Secondly when the cells are in the dormant phase, they accumulate lipids. This latter finding is nothing different from what was already known in the prior art. However, in the present invention, Applicants show that positively applying a growth-limiting stress factor to the cell culture at the end of the exponential phase causes the cells to produce polyunsaturated fatty acids. Applicants direct the Examiner's attention to page 266, left column, 2nd paragraph, wherein it is mentioned that "remaining low concentrations of nitrate and phosphate keep algae alive to produce more lipids per cell even when algae cannot grow further due to silicate exhaustion from medium". From this quotation, it is clear that the media is not actively deprived of silica, but simply that the media runs out of silica naturally and that no further silica has been added. Accordingly, Taguchi does not teach the steps of the claimed method.

Therefore the rejection under 35 U.S.C. 102(b) for anticipation by Taguchi is improper and should be withdrawn.

The rejection of claims 1-6, and 11 under 35 U.S.C. 103(a), for obviousness are respectfully traversed.

Regarding the Examiner's rejection of claims 1-6 and 11 as allegedly obvious over McGinnis et al., taken with Dempster and Taguchi et al., Applicants reiterate that none of the references specifically teach a method of producing polyunsaturated fatty acids in diatomaceous algae. All the references cited by the Examiner only teach a method of producing lipids. There is no teaching or suggestion in any of the cited references that specifically, polyunsaturated fatty acids can be enriched. Further, the references cited by the Examiner are not enabling for a method of specifically increasing polyunsaturated fatty acids. Applicants wish to remind the Examiner that lipids encompass a broad class of molecules such as fats, oils, waxes, cholesterol, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. Thus, demonstrating that the lipid content is increased does not necessarily mean that the polyunsaturated fatty acids content is increased. For example, the content of vitamins might increase, which will translate in an increase of the total lipids amount, without affecting the amount of polyunsaturated fatty acids. It is thus believed that the claims now on file are novel and inventive in view of the prior art, taken alone or combination.

Further, McGinnis points out that research results in certain species are not observed in other species in the same study (McGinnis page 23, 2nd para.). And, as stated in Taguchi (page 260, column 2, 1st para):

> "The production and storage of lipids by microalgea are regulated by environmental factors in a manner that is not always systematic, and can be very species specific. Despite years of research … few accurate generalizations have emerged…."

Therefore, the references alone and in combination do not teach or suggest the method of the instant application, nor, for the reason stated herein, do any of the references cited render the presently claimed invention obvious. Therefore, the rejection of claims 1-6, and 11 under 35 U.S.C. 103(a), for obviousness is improper and should be withdrawn.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

The Commissioner is hereby authorized to charge any fees and credit any overpayments that may be due in connection with this submission to Nixon Peabody LLP Deposit Account No. 50-0850.

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Respectfully submitted,

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